Submitted 8.9.09 | Revision Received 11.11.09 | Accepted 11.16.09

Determination of Low Density Lipoprotein Particle Size by Polyacrylamide Gradient Gel Electrophoresis in Patients with Coronary Artery Stenosis

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DOI: 10.1309/LMR1MWBGGI3KXZEI

Abstract

Many studies have recently shown that coronary artery disease (CAD) risk was increased 2 to 3 fold in patients with small dense LDL (sdLDL). Therefore, the aim of this study was the evaluation of LDL particle size in patients with coronary artery stenosis and healthy individuals. This is a cross-sectional and case-control study. Eighty-six patients with coronary artery stenosis, 35 patients without coronary artery stenosis identified by angiography, and 30 healthy individuals were studied. LDL particle sizes were measured using 2%–16% polyacrylamide gradient gel electrophoresis. All values are expressed as the mean ±SD. One-way analysis of variance (ANOVA) was used to compare mean values among groups. In this study, mean LDL particle size was significantly smaller in patients with coronary artery stenosis (25.29 nm ± 0.38 nm) than patients without coronary artery stenosis (25.63 nm ± 0.2 nm) and healthy individuals (25.95 nm ± 0.4 nm) ($P<0.001$). The results of this study have shown that sdLDL can increase the risk of coronary artery stenosis.

Elevated levels of LDL cholesterol are a major risk factor for coronary artery stenosis;[1] however, in many studies it was shown that patients with coronary artery stenosis have a normal range of LDL-cholesterol levels.[2] LDL particles are known as heterogeneous particles due to their size, density, and lipid composition.[3] Two evident phenotypes were recognized for LDL particles using gradient gel electrophoresis. Phenotype A consists of large, buoyant LDL particles with a size of more than 25.5 nm, and phenotype B is comprised of small dense LDL (sdLDL) and is 25.5 nm or less.[4,5]

Small dense LDLs are considered to be atherogenic because they readily penetrate the arterial wall and have a low affinity for LDL receptor. They are also susceptible to oxidation. In many studies, it was shown that coronary artery disease (CAD) risk was increased 2 to 3 fold in patients with sdLDL.[6-8] Therefore, sdLDL is considered as a new marker for coronary artery stenosis.[9]

The standard method for separation of LDL particles is ultracentrifugation. Nuclear magnetic resonance (NMR) can also be used for determination of LDL diameter and concentration.[5] However, gradient gel electrophoresis is often used for LDL particle size measurement.[10,11]

The purpose of the present study is to investigate if the LDL particle size can be a better risk marker for coronary artery stenosis than other traditional markers.

Materials and Methods

This is a cross-sectional and case-control study. We studied an Iranian population that included 86 non-diabetic patients with coronary artery stenosis, 35 non-diabetic patients without coronary artery stenosis (who had the symptoms of stenosis) identified by angiography, and 30 healthy individuals without a history of diabetes and hypertension.

LDL particle size was measured using 2%–16% polyacrylamide gradient gel electrophoresis and Tris-base (90M), Boric acid (80M), and Na2EDTA (0.003M) buffer, PH 8.3. Serum samples were mixed in a 4:1 ratio with a solution consisting of 40% sucrose and 0.05% bromophenol blue, and 10 µL of samples were loaded to gels. Three standards were applied, such as thyroglobulin (sigma) and bovine albumin (Merck, Whitehouse Station, NJ) (2 mg/mL) with diameters of 17 nm and 7.1 nm, and serum VLDL with an approximate diameter of 34 nm.[12] Five µL of thyroglobulin was also added to each serum sample as an internal standard. Gels were prerun for 15 minutes at 125 V. After loading samples, gels were run for 15 minutes at 75 V, 1 hour at 200 V, and eventually 30 minutes at 400 V in 10°C-15°C. After electrophoresis, gels were stained with Coomassie Brilliant Blue (CBB) R-250. Coomassie Brilliant Blue stains protein standards, serum proteins, and protein portion (apolipoprotein) of lipoproteins. To distinguish LDL, similar samples were mixed with Sudan Black (100 mg Sudan Black in 20 mL ethylene glycol) before electrophoresis or were stained with Sudan Red (20 mg Sudan Red in 20 mL methanol and 4 mL 0.1M NaOH) after electrophoresis, by the remaining gel in Sudan Red for 24 hours. Comparison of these gels revealed the LDL band. LDL particle sizes were determined by using a calibration curve on the basis of their migration on the gel. The calibration curve was plotted by knowing 3 standards of migration distances on the gel and their size.

HDL and LDL cholesterol were measured by a homogenous method (respectively, [LDL, VLDL, and Chylomicrons were eliminated by reagent 1 (anti-human Beta Lipoprotein antibodies) then reagent 2 was added and HDL-C was determined by CHOD-PAP method] and [HDL, VLDL, and Chylomicrons were eliminated by reagent 1 (Cholesterol ester hydrolase, Cholesterol oxidase, and Catalase) and the LDL was protected. Subsequently, reagent 2 was added, and LDL

was determined by CHOD-PAP method]) (Pars Azmoon, Tehran, Iran). The levels of plasma cholesterol and triglyceride were determined using colorimetric enzymatic method (respectively, [Cholesterol oxidase + 4-aminoantipyrine + phenol] and [glycerol phosphate kinase + amino-antipyrine + 4chlorophenpl]) (Pars Azmoon). All values were measured using UV/Visible Spectrophotometer (Pharmacia, Biotech, Ultraspec 3000, Sparta, NJ).

All values are expressed as the mean ±SD. One-way analysis of variance (ANOVA) were used to compare mean values among groups. A statistically significant difference was defined as $P<0.05$.

Table 1_Baseline Characteristics of the Subjects

Variables	*P*	With Stenosis n=86	Without Stenosis n=35	Healthy n=30
Sex (female, male)		(31/55)	(21/14)	(15/15)
Age (y)	0.038	56.28 ± 9.4	54.66 ± 10.8	50.69 ± 10.2
LDL-C (mg/dL)	0.583	119.47 ± 16.6	116.11 ± 17.0	118.83 ± 13.3
HDL-C (mg/dL)	0.000	37.05 ± 6.4	43.17 ± 6.8	47.57 ± 10.4
Triglyceride (mg/dL)	0.002	177.70 ± 62.6	141.21 ± 39.4	153.3 ± 28.4
Total cholesterol (mg/dL)	0.522	177.52 ± 44.4	168.91 ± 35.7	178.13 ± 28.6

Values are mean ±SD

Results

In this study, the serum triglyceride level was higher, but the serum HDL cholesterol was lower in patients with coronary artery stenosis than other groups. However, the serum LDL cholesterol level had no significant difference among groups (**Table 1**).

As shown in **Image 1**, smaller LDL particles had more electrophoretic movement in the gradient gel because of their smaller size.

The diameter of LDL particles was measured by calibration curve on the basis of their electrophoretic movement (**Figure 1**).

In this study, mean LDL particle size was significantly smaller in patients with coronary artery stenosis (25.29 nm ± 0.38 nm) than patients without coronary artery stenosis (25.63 nm ± 0.2 nm) and healthy individuals (25.95 nm ± 0.4 nm) ($P<0.001$).

Discussion

The results of the current study confirm that LDL particle size should be considered as an important risk factor for coronary artery stenosis. The theory of sdLDL and CAD was studied in many retrospective[4,13] and prospective[14,15] studies. In addition, Shoji and colleagues have indicated that sdLDL can be the best marker of carotid atherosclerosis.[16]

Increased levels of LDL cholesterol are a major risk factor for CAD,[1] however, in many studies it was shown that patients with CAD have a normal range of LDL cholesterol levels.[2] In our study, patients with coronary artery stenosis had a normal range of LDL cholesterol levels, too. Griffen and colleagues reported that the elevated level of LDL up to a triglyceride value of 1.5 mmol/l was because of an increase in the buoyant subclass. About 1.5 mmol/l sdLDL was shown to increase significantly, while the concentration of the large and buoyant LDL dropped, resulting in a decrease of total LDL.[17] Therefore, an elevated level of sdLDL can be an important risk factor for artery stenosis.

In the present study, patients with coronary artery stenosis the size of the LDL particles were smaller, so our results are in agreement with the study of Koba,[7] Yoon,[18] St-Pierre,[19] Liu,[20] Kamigaki,[21] Skoglund-Anderson,[22] and Kwon.[23] However, in the Hulthe[24] study, large LDL was correlated with

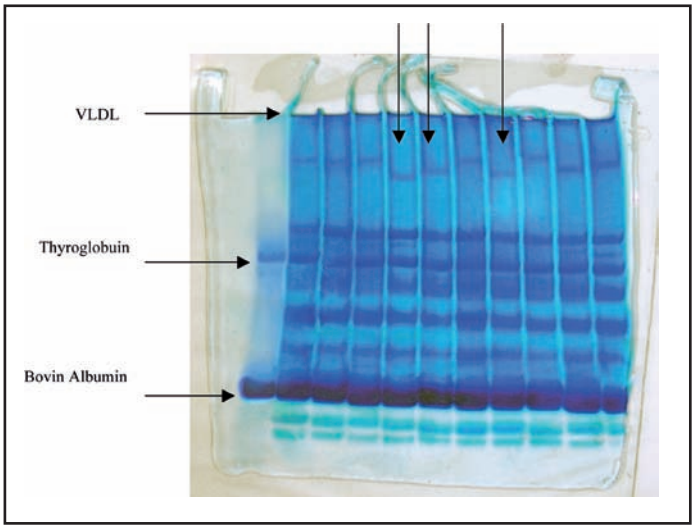

Image 1_Gel stained by CBB. Arrows show the lanes with smaller LDL particle size.

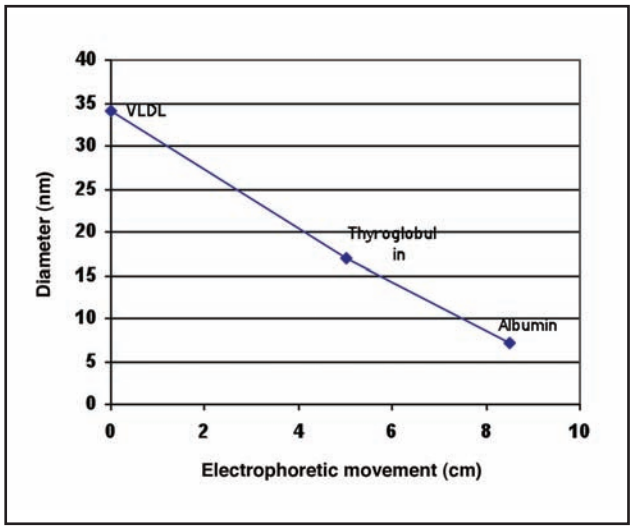

Figure 1_Calibration curve plotted using 3 standards with known diameters, such as thyroglobulin (17 nm), bovin albumun (7.1 nm), and VLDL (34 nm) and their movement.

CAD, and in the Gray study there was no difference in LDL particle size between CAD patients and healthy individuals. Large LDL is common among the Native American society where the CAD prevalence is high.[24] Large LDL is current in countries with high prevalence of CAD such as Scotland, Korea, and the United States;[5] therefore, ethnic and genetic agents may have an important role. Many studies have revealed that sdLDL particles can intensify the atherosclerotic process due to their penetration of the arterial wall, less affinity for LDL receptor, and susceptibility to oxidation.[26]

Our previous study showed that patients with coronary artery stenosis have higher levels of sdLDL than patients without coronary artery stenosis and healthy individuals.[27] According to our previous study, LDL particle size is negatively correlated with sdLDL levels. Therefore, patients with higher levels of sdLDL have a smaller LDL size. Hirano and colleagues have shown that sdLDL levels correlated with LDL size.[10]

Our results indicated that patients with coronary artery stenosis have elevated serum triglyceride levels and reduced HDL cholesterol levels. Serum triglyceride is a primary factor in sdLDL production. In this study, patients with coronary artery stenosis had higher levels of serum triglyceride. Increased levels of serum triglyceride leads to VLDL1 accumulation, due to increased production and reduced clearance of VLDL1. VLDL1 lypolisis produces LDL particles which are unable to attach to LDL receptors due to Apo B100 conformational changes so its retention time is longer than LDLs, which are produced by VLDL2.[28] This form of LDL has sufficient time to be altered by CETP. Therefore, in a VLDL1 level elevation, LDL will lose cholesteryl ester and obtain triglyceride through CETP mechanism. Triglyceride-rich LDL is a good substrate for hepatic lipase. Hepatic lipase withdraws enough amounts of core lipid (triglyceride) and the superficial lipid, and can shift LDL particles toward sdLDL particles.[26] Hence, it is considerable that increased serum triglyceride can lead to sdLDL production in patients with coronary artery stenosis.

In conclusion, we demonstrate that patients with coronary artery stenosis have smaller LDL particles, so sdLDL can increase the risk of coronary artery stenosis. LM

Acknowledgments: The authors thank the financial support of Iran University of Medical Sciences. Our thanks also go to the staff of the Cellular and Molecular Research Center of Iran University of Medical Sciences.

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